

Claims

1. A purified aldehyde dehydrogenase having the following physico-chemical properties:
 - a) Molecular weight of $190,000 \pm 15,000$ Da (consisting of a subunit structure of two α subunits and one β subunit) or molecular weight of $250,000 \pm 20,000$ Da (consisting of a subunit structure of two α subunits and two β subunits), wherein the α subunit has a molecular weight of $75,000 \pm 3,000$ Da and the β subunit has a molecular weight of $55,000 \pm 2,000$ Da;
 - b) Substrate specificity: active on aldehyde compounds,
 - 10 c) Cofactors: pyrroloquinoline quinone (PQQ) and heme c,
 - d) Optimum pH: from about 6.5 to about 8.0 (for the production of vitamin C from L-sorbose) or about 9.0 (for the production of 2-keto-L-gulonate from L-sorbose),
 - e) Inhibitors: Co^{2+} , Cu^{2+} , Fe^{3+} , Ni^{2+} , Zn^{2+} , Mg^{2+} , monoiodoacetate and sodium
15 azide.
2. The aldehyde dehydrogenase according to claim 1, which is derived from a microorganism belonging to the genus *Gluconobacter* which is capable of producing said aldehyde dehydrogenase.
3. The aldehyde dehydrogenase according to claim 2, wherein the microorganism is
20 *Gluconobacter oxydans* having the identifying characteristics of the strain *Gluconobacter oxydans* DSM No. 4025 (FERM BP-3812), a subculture or mutant thereof.
4. The aldehyde dehydrogenase according to claim 3, wherein the microorganism is *Gluconobacter oxydans* DSM No. 4025 (FERM BP-3812), a subculture or mutant thereof.
5. A process for producing an aldehyde dehydrogenase having the following physico-
25 chemical properties:
 - a) Molecular weight of $190,000 \pm 15,000$ Da (consisting of a subunit structure of two α subunits and one β subunit) or molecular weight of $250,000 \pm 20,000$ Da (consisting of a subunit structure of two α subunits and two β subunits), wherein the α

subunit has a molecular weight of $75,000 \pm 3,000$ Da and the β subunit has a molecular weight of $55,000 \pm 2,000$ Da;

- b) Substrate specificity: active on aldehyde compounds,
- c) Cofactors: pyrroloquinoline quinone (PQQ) and heme c,
- 5 d) Optimum pH: from about 6.5 to about 8.0 (for the production of vitamin C from L-sorbose) or about 9.0 (for the production of 2-keto-L-gulonate from L-sorbose),
- e) Inhibitors: Co^{2+} , Cu^{2+} , Fe^{3+} , Ni^{2+} , Zn^{2+} , Mg^{2+} , monoiodoacetate and sodium azide,
- 10 which comprises cultivating a microorganism belonging to the genus *Gluconobacter*, which is capable of producing the aldehyde dehydrogenase having the above properties, in an aqueous nutrient medium under aerobic conditions, disrupting the cells of the microorganism, and isolating and purifying the aldehyde dehydrogenase from the cell-free extract of the disrupted cells of the microorganism.
- 15 6. The process according to claim 5, wherein the reaction is carried out at a pH of from about 4.5 to about 9.0 and at a temperature of from about 20 to about 50°C.
- 7. A process for producing a carboxylic acid and/or its lactone from its corresponding aldose which comprises contacting the aldehyde with the purified aldehyde dehydrogenase having the following physico-chemical properties:
- 20 a) Molecular weight of $190,000 \pm 15,000$ Da (consisting of a subunit structure of two α subunits and one β subunit) or molecular weight of $250,000 \pm 20,000$ Da (consisting of a subunit structure of two α subunits and two β subunits), wherein the α subunit has a molecular weight of $75,000 \pm 3,000$ Da and the β subunit has a molecular weight of $55,000 \pm 2,000$ Da;
- 25 b) Substrate specificity: active on aldehyde compounds,
- c) Cofactors: pyrroloquinoline quinone (PQQ) and heme c,
- d) Optimum pH: from about 6.5 to about 8.0 (for the production of vitamin C from L-sorbose) or about 9.0 (for the production of 2-keto-L-gulonate from L-sorbose),

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e) Inhibitors: Co^{2+} , Cu^{2+} , Fe^{3+} , Ni^{2+} , Zn^{2+} , Mg^{2+} , monoiodoacetate and sodium azide,

or cell-free extract prepared from a microorganism belonging to the genus *Gluconobacter* which is capable of producing the aldehyde dehydrogenase having the above properties in
5 the presence of an electron acceptor.

8. The process according to any one of claims 5 to 7, wherein the microorganism is *Gluconobacter oxydans* having the identifying characteristics of the strain *Gluconobacter oxydans* DSM No. 4025 (FERM BP-3812), a subculture or mutant thereof.

9. The process according to claim 8, wherein the microorganism is *Gluconobacter*
10 *oxydans* DSM No. 4025 (FERM BP-3812), a subculture or mutant thereof.

10. The process of claim 7, wherein the lactone is vitamin C, the carboxylic acid is 2-keto-L-gulonic acid and the aldose is L-sorbose.

11. The process according to any one of claims 7 to 10, wherein the reaction is carried out at a pH of from about 4.5 to about 9.0 and at a temperature of from about 20 to
15 about 50°C for the production of vitamin C and 2-keto-L-gulonic acid, respectively.

12. The process according to any one of claims 7 to 11, wherein the reaction is carried out at a pH of from about 6.5 to about 8.0 for vitamin C production and of about 9.0 for 2-keto-L-gulonic acid production, and at a temperature of from about 20 to about 50°C for both production ways.

20 13. The use of the purified aldehyde dehydrogenase of claim 1 in the process for the production of a carboxylic acid and/or its lactone from its corresponding aldose which comprises contacting the aldehyde with said purified aldehyde dehydrogenase or cell-free extract prepared from a microorganism belonging to the genus *Gluconobacter* which is capable of producing said aldehyde dehydrogenase in the presence of an electron
25 acceptor.
